



Figures 30a and 30b show the identification of the coimmunoprecipitated proteins.

Figures 31a-c show an analysis of the association of 4-1BB and p56^{lck} in a baculoviral expression system.

5 Figures 32a-c show an analysis of the association of 4-1BB and p56^{lck} HeLa cells.

Figure 33 is a Western analysis that shows the expression of the 4-1BB-AP fusion protein and rs4-1BB.

10 Figures 34 a-c show the quantitative analysis of 4-1BB-AP binding to lymphoid- and nonlymphoid-cell lines.

Figures 35a and 35b show the characterization of 4-1BB-AP binding to A20 B-cell lymphoma cells.

Figure 36 shows the costimulation of anti-r-primed B cells with fixed-SF21-4-1BB cells.

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DETAILED DESCRIPTION

20 In the following detailed description a successive series of studies are presented which characterize the receptor 4-1BB. References are made to known procedures and studies, as well as published work of the applicant. These publications are incorporated herein by reference for clarity and listed in an appendix included at the end of this detailed description.

25 The following abbreviations are used herein: CTL, cytolytic T lymphocyte; HTL, helper T lymphocyte; LGL, large granular lymphocytes; NK, natural killer cells; SDS, sodium dodecylsulfate; SSC, 150 mM sodium chloride/15 mM sodium citrate, pH 7.0; TPA, 12-O-tetradecanoylphorbol-13-acetate. Th, helper T lymphocytes; IL-2, interleukin 2; IL-3, interleukin 3; rIL-2, recombinant IL-2; CSF-30 GM, granulocyte/macrophage colony-stimulating factors; cRNA, complementary RNA; ss, single-stranded; ds, double-stranded; TCR, T-cell antigen receptor; PTA, phorbol 12-tetradecanoate 13-acetate; r, recombinant; mu, murine; hu, human; MIP,

demonstrated that 4-1BB specifically associated with p56^{lck} and excluded the possibility that other T-cell factors were required for the interaction.

The interaction between CD4 or CD8 and p56^{lck} requires a specific binding site on each molecule^{2,3}. The N-terminal region of p56^{lck} interacts noncovalently with the cytoplasmic domains of CD4 and CD8 via pairs of cysteine residues in each molecule. Therefore, it was determined whether the same cysteines of p56^{lck} (cysteine 20 and 23) are required for the association with 4-1BB. p56^{lck} constructs containing sequences encoding mutated p56^{lck} proteins (construct C₁, cys 20- ser; construct C₂, cys 23- ser)² were employed in the following experiments. The capability of the cysteine-mutant p56^{lck} proteins to associate with 4-1BB was tested by the coimmunoprecipitation assay. A vaccinia virus expression system was utilized in which the T7 RNA polymerase-expressing vaccinia virus allows transfected genes under the control of the T7 promoter to be expressed in HeLa cells². High levels of coexpression have been achieved previously with this system to facilitate interactions between CD4 and p56^{lck}. To ensure consistent expression of 4-1BB, the transfected HeLa cells were briefly labeled with [³⁵S] cysteine before harvest. 4-1BB expression was monitored by immunoprecipitation of ³⁵S-labeled 4-1BB with 53A2.

Figures 32a-c show an analysis of the association of 4-1BB and p56^{lck} HeLa cells. Figure 32a shows an immunoblot of p56^{lck} HeLa cells were transfected with cDNAs encoding the indicated proteins and metabolically labeled with [³⁵S] cysteine. Lysates were immunoprecipitated with antibodies to p56^{lck} and 4-1BB and labeled with [^γ-³²P] ATP by the in vitro kinase reaction. The positions of p56^{lck} and 4-1BB are indicated to the right. Total HeLa cell lysates were immunoblotted with antibody to p56^{lck} assay the p56^{lck} expression. Figures 32b and 32c show an immune complex kinase assay. Aliquots of the saline lysates were immunoprecipitated with anti-p56^{lck} serum (Fig. 32b) or 53A2 (Fig. 32c) followed by the in vitro kinase reaction with [^γ-³²P] ATP as described in the legend to Fig. 30. 1 x 10⁶ HeLa cells were grown to 80% confluency on 100-mm petri dishes in DMEM containing 10% FBS and antibiotics. Cells were infected with 1 x 10⁸ pfu of vaccinia virus expressing T7 RNA polymerase (136) 30 min before transfection. DNA was transfected in liposomes (Lipofectin, GIBCO, BRL). 15 μg DNA and 50 μl liposome diluted into 5 ml